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## Synthesis and evaluation of [18F]fluoroproggestins and [18F]fluorometoprolol

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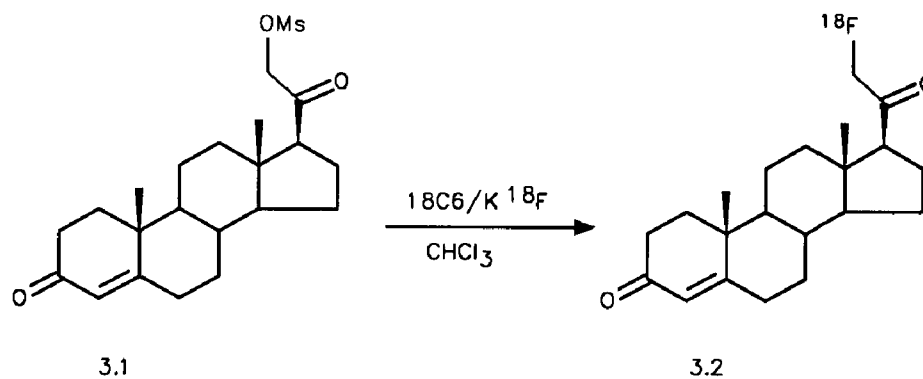
## CHAPTER 3

### SYNTHESIS OF 21- $^{18}\text{F}$ FLUORO-PROGESTINS

*A review is given on the synthesis of 21- $^{18}\text{F}$ fluorinated steroids for the in vivo visualisation of progesterone receptors. The no carrier added synthesis of 16 $\alpha$ -alkylated 21- $^{18}\text{F}$ fluoro-progestins, a class of potential tracers for the progesterone receptor, is described in detail and the results of an in vivo evaluation of two of the most promising ligands are discussed.*

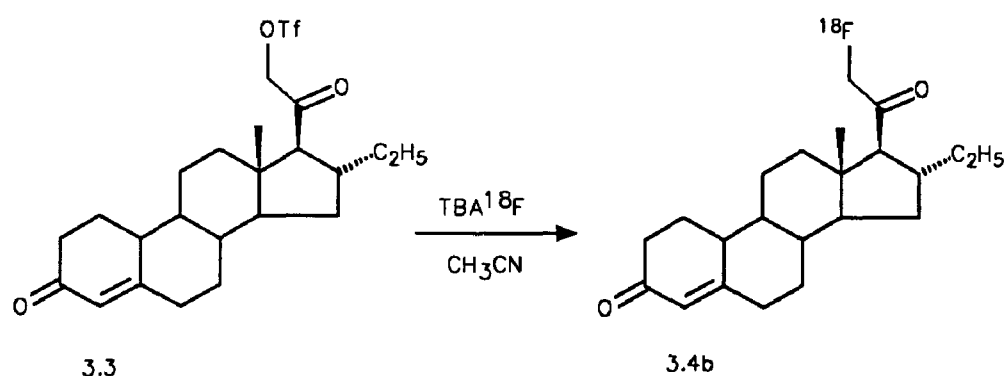
#### 3.1 21-Fluoro-progestins in literature

Irie *et al.*<sup>108</sup> and Spitznagle and Marino<sup>110</sup> have reported the synthesis of 21- $^{18}\text{F}$ fluoroprogestosterone **3.2**. This compound was prepared from the corresponding 21-mesylate **3.1** with a 18-crown-6/ $\text{K}^{18}\text{F}$  complex in 20-40% yield [corrected for decay to end of bombardment (EOB), Scheme 3.1]. The relatively high yield of the reaction can be explained by the addition of carrier KF, resulting in a low specific activity of **3.2**. In a no carrier added (n.c.a.) synthesis a radiochemical yield of 10-14% (EOB) was reported.<sup>111</sup>



Scheme 3.1

During our research on the synthesis of 21- $^{18}\text{F}$ fluoro-16 $\alpha$ -alkyl substituted progestins,<sup>112</sup> Pomper *et al.*<sup>113</sup> reported the synthesis of 21- $^{18}\text{F}$ fluoro-16 $\alpha$ -ethyl-19-norprogesterone **3.4b** (Scheme 3.2). They used the corresponding 21-triflate **3.3** as a key intermediate and **3.4b** was prepared in 4-30% radiochemical yield (EOB). No explanation was given for the great variation in yields of **3.4b**.



*Scheme 3.2*

As was mentioned in Chapter 1, throughout the years many progestins have been screened for their progestational activity. One of the compounds that emerged was 21-hydroxy-16 $\alpha$ -ethyl-19-norprogesterone (Org 2058) **3.5b** (Figure 3.1). The tritiated form of **3.5b** (6,7- $^3\text{H}$ ) is nowadays widely used in *in vitro* assays of progesterone receptors (PR). Several affinity studies with **3.5b** and related compounds such as the 21-fluoro analogue **3.4b** have been carried out.<sup>59,114</sup> Some results are shown in Table 3.1.

Compound	RBA <sup>59</sup>	RBA <sup>114</sup>
progesterone	100	38
19-norprogesterone	130	139
16 $\alpha$ -ethylprogesterone	95	46
Org-2058 <b>3.5b</b>	145	100
21-fluoro-16 $\alpha$ -ethyl-19-norprogesterone <b>3.4b</b>	170	147
d-norgestrel <b>3.6</b>	165	81
medroxyprogesterone acetate	196	73

*Table 3.1 Relative binding affinity (RBA) of a selected number of progestins.*

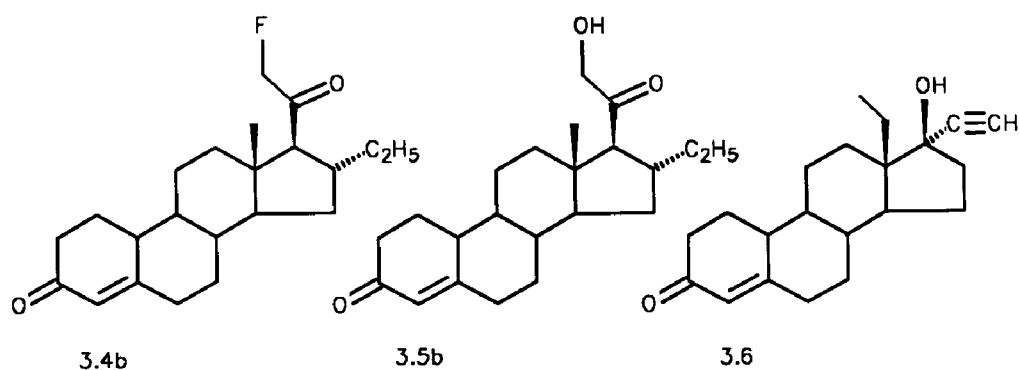


Figure 3.1 Structure of 21-fluoro-16 $\alpha$ -ethyl-19-norprogesterone (FENP, **3.4b**), Org 2058 (**3.5b**) and norgestrel (**3.6**).

The positive influence of a 21-fluorine substitution of progesterone on the affinity for PR has been emphasized in Chapter 1. In addition, the 21-position is readily accessible for a nucleophilic substitution with [ $^{18}\text{F}$ ]fluoride. In consideration of these two aspects, we investigated the synthesis of **3.4b** in a fluorine-18 labelled form. Some analogues of **3.4b** were also prepared in order to investigate the effect of the 16 $\alpha$ -substitution on the binding of the ligand to PR *in vivo*.

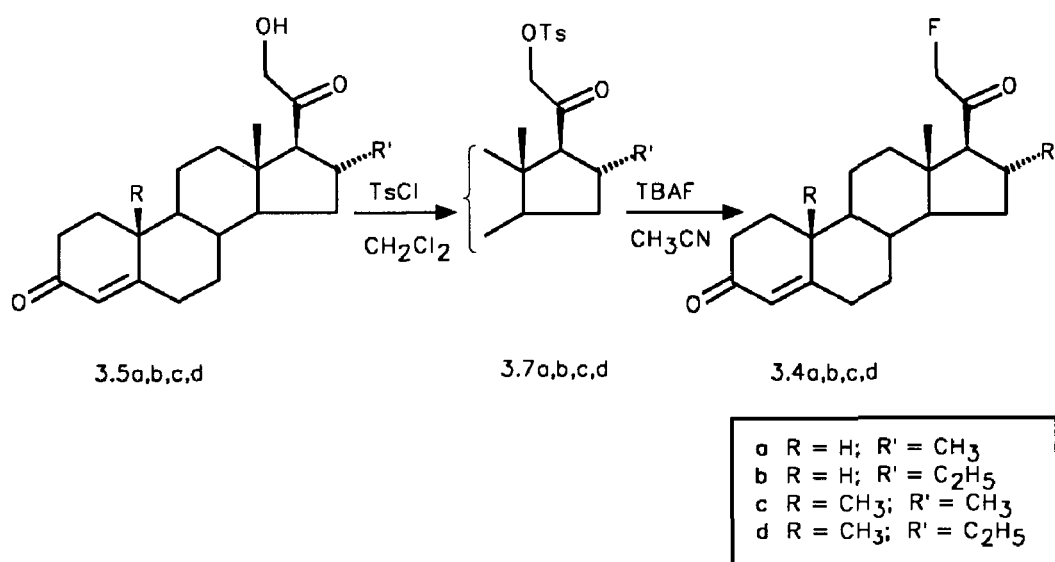
### 3.2 Synthesis of 21-fluoro-progestins

21-Fluoro-16 $\alpha$ -ethyl-19-norprogesterone **3.4b** has been described in the literature and was prepared from the corresponding 21-iodide by a substitution with silver fluoride.<sup>114</sup> An analogue of this compound, 21-fluoro-16 $\alpha$ -methylprogesterone **3.4c** has also been reported previously<sup>115</sup> and was prepared analogously to **3.4b**.

We have chosen to use the tosylate as leaving group in the nucleophilic substitution reaction with [ $^{18}\text{F}$ ]fluoride because of its stability and excellent leaving group ability. Sulfonic esters are very reactive towards nucleophilic substitution and their use is widespread in organic chemistry. The triflate (trifluoromethanesulfonate) and the tosylate (*p*-toluenesulfonate) are among the most reactive leaving groups known<sup>116</sup> and have been used successfully in the synthesis of various fluorine-18 labelled compounds. Both 1, $\omega$ -ditosyloxy-alkanes

as well as 1, $\omega$ -ditriflates have been used in the preparation of positron emitting ligands, such as 1,2-ditosyloxyethane in the synthesis of [ $^{18}\text{F}$ ]fluoroethyl-spiperone.<sup>117</sup> Kiesewetter *et al.*<sup>40</sup> have used the triflate as leaving group in the preparation of 16 $\alpha$ -[ $^{18}\text{F}$ ]fluoroestradiol **1.7a**.

The synthesis of the "cold" fluorides **3.4a,b,c,d** is shown in Scheme 3.3. The 21-hydroxy-16 $\alpha$ -alkylated progestins **3.5a,b,c,d** were synthesized according to the method developed by van Leusen<sup>118</sup> and described in his Ph.D. thesis. The 21-tosylates **3.7a,b,c,d** were prepared in 75% yield by the reaction of **3.5a,b,c,d** with *p*-toluenesulfonyl chloride.



Scheme 3.3

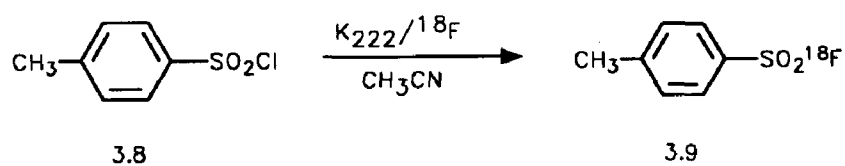
The synthesis of **3.4a,c,d** was accomplished with anhydrous tetrabutylammonium fluoride (TBAF) in acetonitrile in 30% yield (Scheme 3.3). Compounds **3.4a,b,c,d** were used to ascertain the identity and purity of the [ $^{18}\text{F}$ ]fluoro analogues **3.4a,b,c,d**. The preparation of the radioactive compounds will be described in the next section.

### 3.3 Synthesis of 21-[ $^{18}\text{F}$ ]fluoro-progestins

In Chapter 2, the targets and the work-up procedure for [ $^{18}\text{F}$ ]fluoride were described. The Kryptofix 222/ $\text{K}_3\text{PO}_4$ /[ $^{18}\text{F}$ ]-complex ( $\text{K}_{222}/^{18}\text{F}$ ) thus obtained, was

used in the preparation of the 21- $^{18}\text{F}$ fluoro-16 $\alpha$ -alkylated progesterone derivatives **3.4a,b,c,d**.

An important aspect in the synthesis of the fluorine-18 labelled **3.4a,b,c,d** was the purity of tosylates **3.7a,b,c,d**. The contamination of **3.7a,b,c,d** with a small amount of *p*-toluenesulfonyl chloride **3.8** suppressed the substitution reaction with  $^{18}\text{F}$ fluoride completely. Instead of substituting the 21-tosylate,  $^{18}\text{F}$ fluoride reacted with **3.8** to yield the volatile *p*-toluenesulfonyl  $^{18}\text{F}$ fluoride **3.9** (Scheme 3.4).

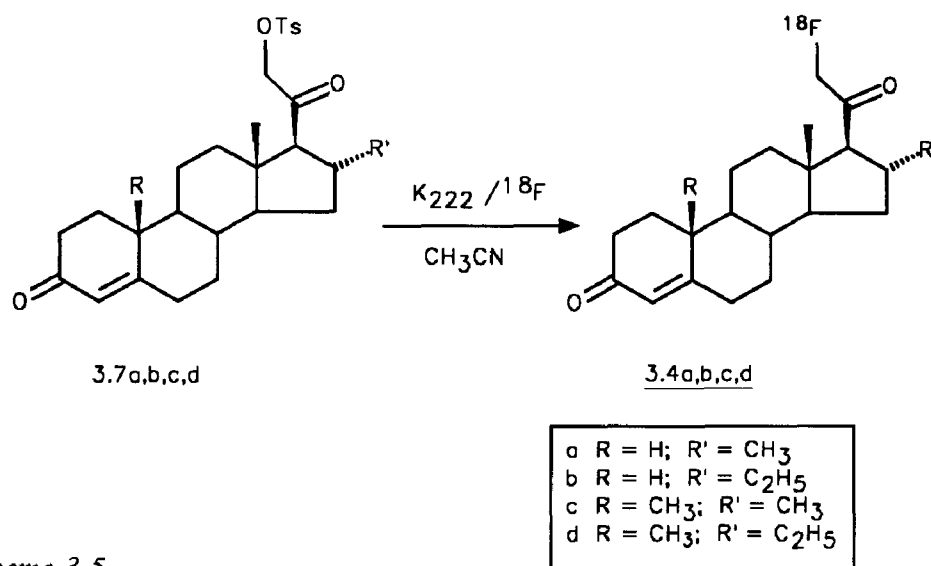


Scheme 3.4

The nucleophilic substitution of **3.7b** with Kryptofix 222/KF was investigated under semi-preparative conditions in several solvents, with 0.5 eq.  $\text{F}^-$  with respect to the tosylate. With Kryptofix 222, 0.5 eq.  $\text{K}_2\text{CO}_3$  was added to the reaction mixture. It was not possible to mimic the actual radioactive substitution reaction with  $^{19}\text{F}^-$  due to the extremely high substrate-fluoride ratio ( $>10^4$ ) in the reactions with  $^{18}\text{F}$ fluoride. However, the results of these experiments gave a good indication of which conditions were most appropriate for the reaction with  $^{18}\text{F}$ fluoride. From Table 3.2 it can be observed that the synthesis of **3.4b** in acetonitrile gave the best result. This finding is in accordance with the experience acquired with many other nucleophilic substitutions with  $^{18}\text{F}$ fluoride.<sup>84</sup>

solvent	T (°C)	time (min)	yield (%)
$\text{CH}_3\text{CN}$	25	70	30
$\text{CH}_3\text{CN}$	100	70	90
DMF	100	30	20
DMSO	100	30	30

Table 3.2 Yields of the conversion of **3.7b** to **3.4b**.



*Scheme 3.5*

The reaction of **3.7b** with  $\text{K}_{222}/^{18}\text{F}$  was monitored in acetonitrile at a temperature of 70 °C. The employed reaction temperature was lower than the optimum of 100 °C found in the "cold" experiments described above, because the tosylate was not stable under the conditions required for the radioactive reaction. The base/fluoride ratio is much higher in the reaction with [ $^{18}\text{F}$ ]fluoride and the tosylate is probably saponified by the excess of base. This explanation is confirmed by the fact that the amount of salt used in the resolubilisation of the [ $^{18}\text{F}$ ]fluoride is very critical. A larger amount than 30  $\mu\text{mol}$  Kryptofix 222/ $\text{K}^+$  (in 0.5 ml acetonitrile) should be avoided in the reaction procedure, otherwise the yield of the substitution reaction is reduced dramatically.

The formation of **3.4b** was monitored by TLC (Figure 3.2). After a rapid increase of the yield of **3.4b** in the first 2-5 minutes, a plateau was reached. The optimum reaction time is 5-10 min. In this reaction, with a high tosylate/[ $^{18}\text{F}$ ]fluoride ratio, a marked increase of the yield of **3.4b** was found when the amount of tosylate was increased from 1 mg to 2-3 mg in 0.5 ml acetonitrile (6% and 20% EOB, respectively). However, the application of more than 1 mg **3.7a,b,c,d** interfered with the isolation of the [ $^{18}\text{F}$ ]fluorinated products by HPLC. The separation of **3.4a,b,c,d** from its byproducts is very critical as is illustrated by Figure 3.3. Therefore, in the synthesis of **3.4a,b,c,d**, applied for patient studies and animal experiments, an amount of 1 mg of **3.7a,b,c,d** was used.

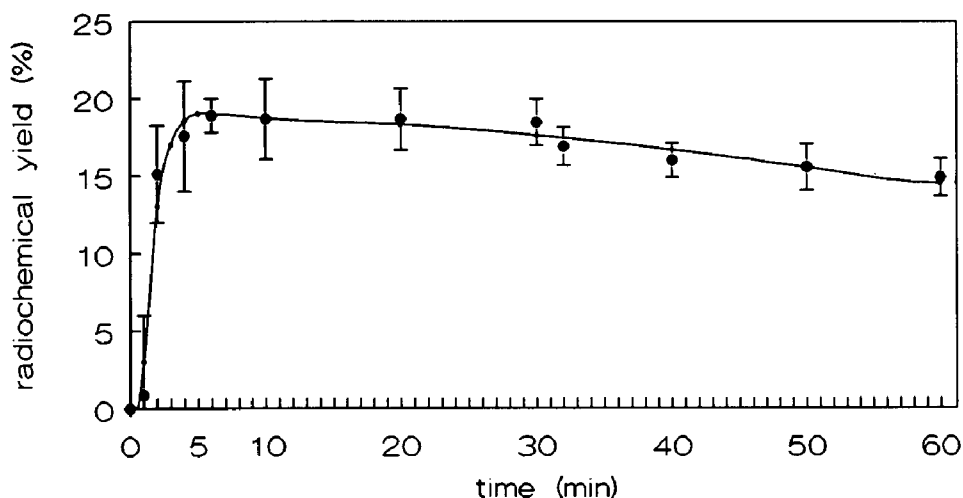


Figure 3.2 Radiochemical yield (EOS, mean  $\pm$  S.D.,  $n=3$ ) of **3.6b** as a function of time. Conditions: 2 mg **3.7b** in 0.5 ml  $\text{CH}_3\text{CN}$ , 70 °C. TLC: silica, eluted with  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  98/2 (v/v),  $R_f=0.9$ .

The HPLC purification of **3.4a,b,c,d** was investigated both on a silica column (system A) and reversed phase (C18 column, system B). The best separation of **3.4a,b,c,d** from the byproducts was found on system A, and this method was used for the routine purification of **3.4a,b,c,d**.

Compound	$t_R$ (min)	
	A	B
<b>3.4a</b> FMNP	27.4	7.3
<b>3.4b</b> FENP	26.0	9.9
<b>3.4c</b> FMP	20.4	8.6
<b>3.4d</b> FEP	16.8	11.6

Table 3.3 Retention times ( $t_R$ ) of **3.4a,b,c,d** on HPLC systems A and B. For details see Section 3.5.

An HPLC-profile of the purification of **3.4a** in a preparative run is shown in Figure 3.3. This typical reaction yielded 22 MBq (0.6 mCi, 8% EOB) of **3.4a**



with a specific activity of 200 GBq/ $\mu$ mol (6,000 Ci/mmol, EOS). An identical profile was found for the other labelled products **3.4b,c,d**. The dramatic effect of the high specific activity is clearly demonstrated by the small amount of mass coeluting with the radioactive product. Because **3.5a** and **3.7a** eluted from the column after product **3.4a**, the major amount of mass is not shown in Figure 3.3. No attempt has been made to identify the "cold" products eluting shortly before **3.4a**.

Pomper *et al.*<sup>113</sup> reported that the separation of **3.4b** from the 21-triflate **3.3** is more troublesome, because **3.3** eluted just before **3.4b** and as a consequence a considerable amount of mass coeluted with **3.4b**. An extra hydrolysis step had to be introduced in the reaction sequence to destroy unreacted **3.3** and attain a satisfactory chemical purity.

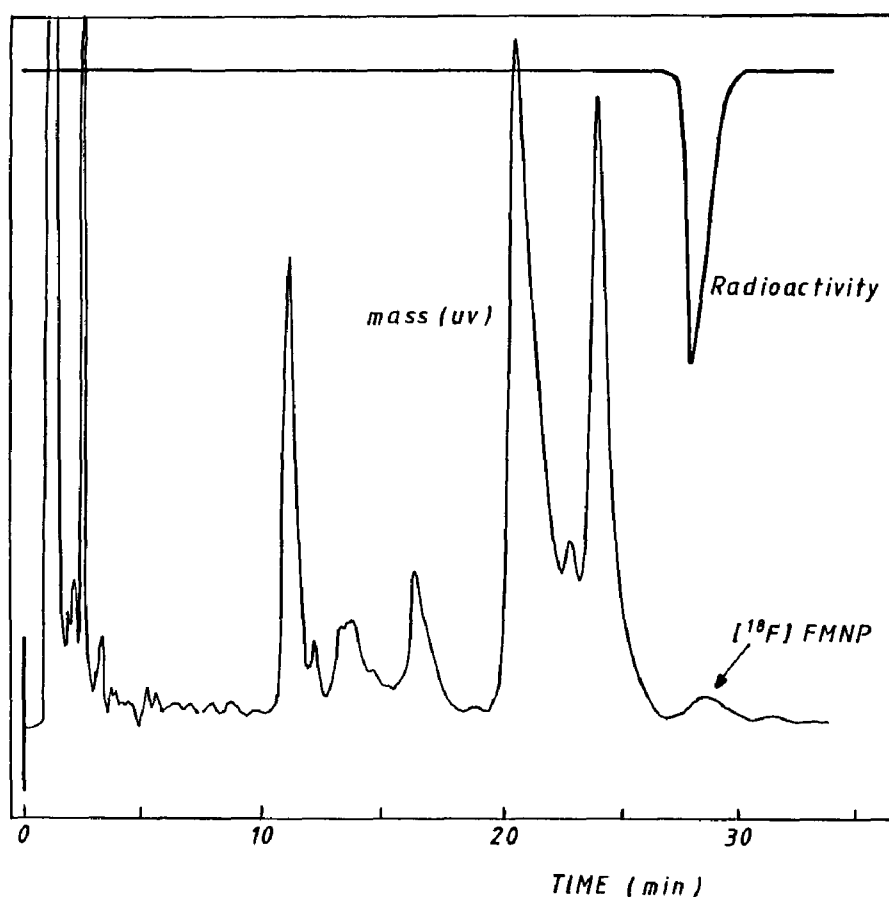


Figure 3.3 HPLC chromatogram of the purification of **3.4a**. (System A, UV absorption units full scale 0.02).

The total synthesis time of **3.4a,b,c,d**, starting from EOB and including HPLC purification, was 100 minutes. The radiochemical yield ranged from 7-14%, with an average of 10% (EOB). In relation to radiochemical yields reported by other authors, the yield of **3.4a,b,c,d** is satisfactory.<sup>111,113</sup> Estimated by UV-absorption, the specific activity of **3.4a,b,c,d** ranged between 200 and 400 GBq/ $\mu$ mol (5,000-10,000 Ci/mmol, EOS). The yield and specific activity of **3.4a,b,c,d** were sufficient for the performance of *in vivo* receptor studies.

### 3.3.1 Remote controlled synthesis

The application of fluorine-18 labelled steroids in patient studies requires 100-200 MBq (3-5 mCi) of [ $^{18}\text{F}$ ]fluorinated product. For the synthesis of 200 MBq [ $^{18}\text{F}$ ]fluoro-progestin an amount of about 4 GBq [ $^{18}\text{F}$ ]fluoride is needed at the beginning of synthesis. In order to reduce the exposure to the radioactivity, a system was developed with which the synthesis could be remotely controlled.

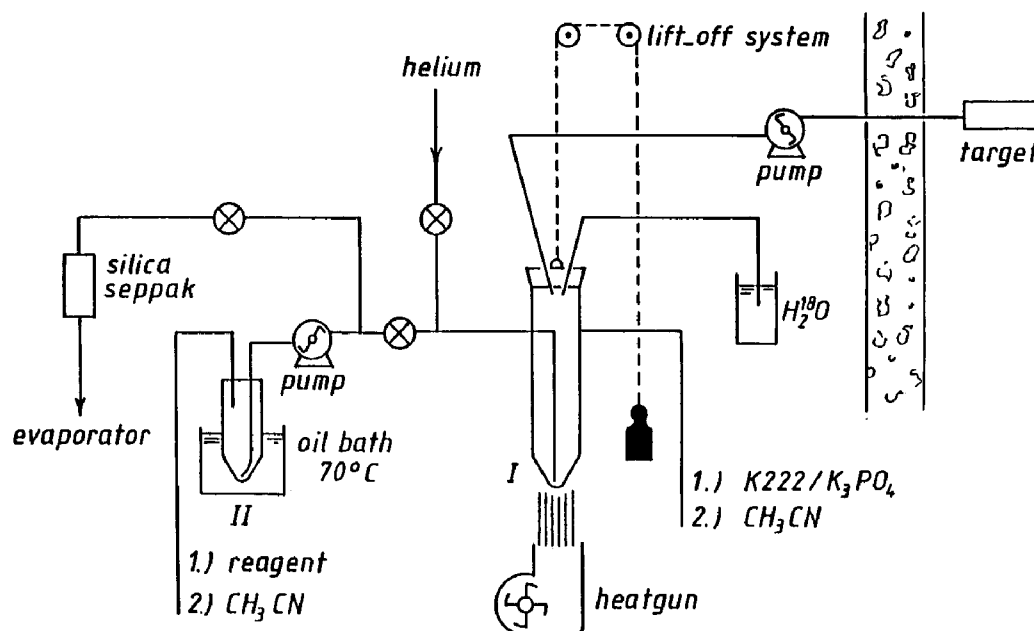


Figure 3.4 Remote controlled set-up for the synthesis of [ $^{18}\text{F}$ ]fluoro-progestins.

Two borosilicate glass vessels were used in the preparation of **3.4a,b,c,d**, vessel I for drying [ $^{18}\text{F}$ ]fluoride and vessel II, a sealed 5 ml conical tube, was used for the actual substitution reaction. A small weight was connected to the cap of vessel I, so the cap could be lifted by gently knocking the vessel with a

remotely operated small hammer (not shown in Figure 3.4). Vessel I was heated by a flow of air (120 °C) to prevent excessive condensation of water at the top of the vessel. Vessel II was heated on an oil-bath.

The transfers of the solutions were achieved with a peristaltic pump and connections were made with silicone and polyethylene tubes. In comparison to the manual procedure, the use of these materials had no detrimental effect on the yield or specific activity of the products. Pinch valves were used to control the flow direction. These valves are very convenient for controlling small volumes, because dead volumes neither are present inside the valve nor occur due to the connections made.

The crude product was separated from unreacted [ $^{18}\text{F}$ ]fluoride with a silica Sep Pak-column, eluted with acetonitrile. The resulting solution was concentrated *in vacuo* by a small rotary evaporator. The purification of the crude product was established with HPLC, as described in section 3.5.

### 3.4 Biodistribution study with 3.4a ([ $^{18}\text{F}$ ]FMNP) and 3.4b ([ $^{18}\text{F}$ ]FENP)

From the literature it is known that 19-norproggestins exhibit a higher affinity for the progesterone receptor than their 19-methyl analogues.<sup>119</sup> In this respect, the two [ $^{18}\text{F}$ ]fluorinated 19-norproggestins 3.4a and 3.4b (Figure 3.5) were selected for a tissue distribution study in rats to evaluate the suitability of 3.4a,b for the *in vivo* visualisation of PR.

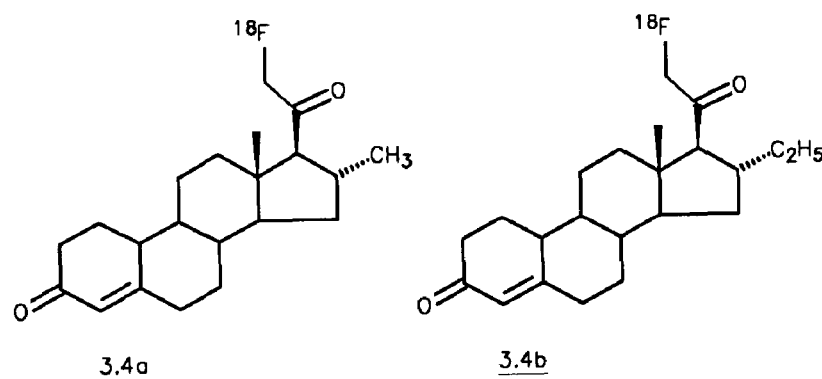


Figure 3.5 Structure of [ $^{18}\text{F}$ ]FMNP 3.4a and [ $^{18}\text{F}$ ]FENP 3.4b.

The results of the distribution studies with the radioligands **3.4a** ( $[^{18}\text{F}]\text{FMNP}$ ) and **3.4b** ( $[^{18}\text{F}]\text{FENP}$ ) have been described by Verhagen *et al.*<sup>120,121</sup> and, independently, for **3.4b** also by Pomper *et al.*<sup>113</sup>

Tissue	60 min	60 min <sup>a</sup>	180 min
Uterus	1.36 ± 0.15 <sup>b</sup>	0.31 ± 0.06	1.45 ± 0.22
Ovaries	0.79 ± 0.13	0.53 ± 0.14	0.54 ± 0.09
Blood	0.20 ± 0.04	0.20 ± 0.04	0.06 ± 0.01
Muscle	0.21 ± 0.05	0.23 ± 0.04	0.08 ± 0.01
Liver	1.47 ± 0.30	1.58 ± 0.30	0.27 ± 0.05
Kidney	0.52 ± 0.08	0.54 ± 0.12	0.16 ± 0.03
Brain	0.25 ± 0.05	0.23 ± 0.04	0.07 ± 0.01
Bone	1.72 ± 0.22	1.81 ± 0.34	2.19 ± 0.38
Fat	0.89 ± 0.19	0.86 ± 0.16	0.96 ± 0.15

<sup>a</sup> + 30 µg FMNP; <sup>b</sup> Mean ± Standard Deviation.

**Table 3.4** Tissue distribution of **3.4a** ( $[^{18}\text{F}]\text{FMNP}$ ) expressed as  $\text{DAR}^{\textcircled{a}}$  after i.v. injection in immature female Wistar rats ( $n=5$ ).<sup>120</sup>

Tissue	60 min	60 min <sup>a</sup>	180 min
Uterus	2.08 ± 0.38 <sup>b</sup>	0.36 ± 0.05	1.69 ± 0.44
Ovaries	1.17 ± 0.27	0.70 ± 0.20	0.65 ± 0.18
Blood	0.15 ± 0.03	0.15 ± 0.02	0.04 ± 0.01
Muscle	0.29 ± 0.05	0.27 ± 0.02	0.11 ± 0.02
Liver	1.59 ± 0.28	1.63 ± 0.20	0.76 ± 0.17
Kidney	0.55 ± 0.06	0.52 ± 0.08	0.23 ± 0.07
Brain	0.36 ± 0.06	0.36 ± 0.05	0.09 ± 0.02
Bone	1.14 ± 0.21	1.24 ± 0.21	1.48 ± 0.20
Fat	1.37 ± 0.30	1.28 ± 0.33	1.57 ± 0.33

<sup>a</sup> + 30 µg FENP; <sup>b</sup> Mean ± Standard Deviation.

**Table 3.5** Tissue distribution of **3.4b** ( $[^{18}\text{F}]\text{FENP}$ ) expressed as  $\text{DAR}^{\textcircled{a}}$  after i.v. injection in immature female Wistar rats ( $n=5$ ).<sup>121</sup>

<sup>ⓐ</sup>  $\text{DAR}$  = Differential absorption ratio, which is calculated from the formula:  
 $\text{DAR} = (\text{radioactivity/g tissue}) \times (\text{g body weight/total injected radioactivity})$ .

The results of the tissue distribution studies are summarized in Tables 3.4 and 3.5. The tissue, which is known to contain a considerable amount of PR (the uterus), is regarded as the target tissue. Muscle is devoid of PR, and hence it is considered as being a non-target tissue. In order to prevent interference of endogenous progesterone with the uptake of **3.4a,b** in the uterus, immature female rats were used in this study. The growth of PR in these animals was induced by three successive, daily injections of estradiol.<sup>122</sup>

The uterus showed a distinct uptake of **3.4a** and **3.4b** (columns 1 and 3 of Tables 3.4 and 3.5). The saturable, receptor mediated uptake of both **3.4a** and **3.4b** was demonstrated by the coinjection of an amount of the corresponding non-radioactive ligand, resulting in a marked decrease in uptake in the uterus, whereas the uptake in non-target tissues is almost unaffected (column 2). The specificity of **3.4a** was demonstrated by coinjection of Org 2058 **3.5b**, a potent, highly selective progestin. This experiment showed a decrease in uptake by the uterus, comparable to the experiments with coinjected unlabelled **3.4a**. The selectivity of **3.4b** has been demonstrated by Kontula *et al.*<sup>59</sup>

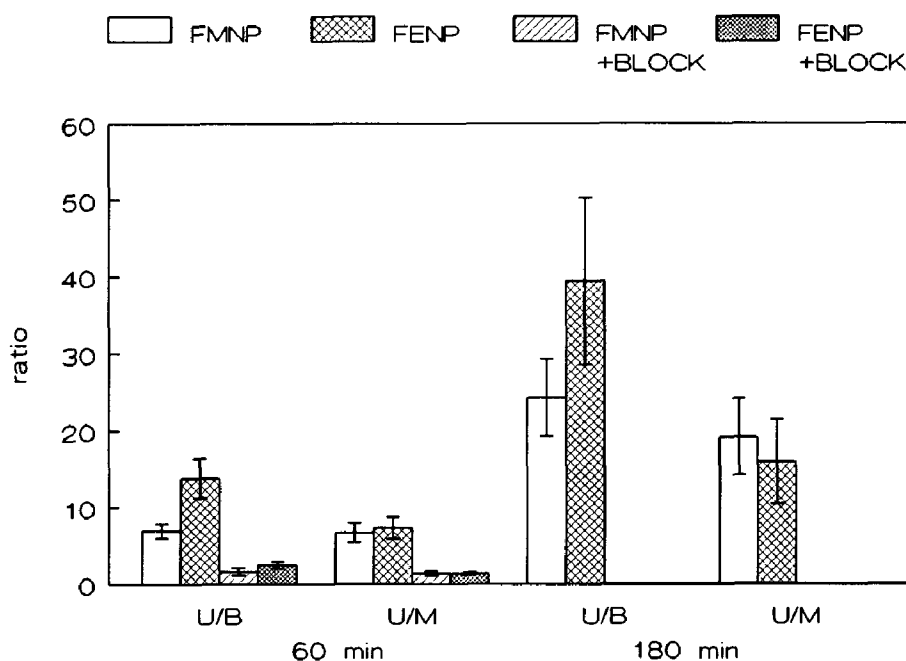


Figure 3.6 Target/non-target ratios for [ $^{18}\text{F}$ ]FMNP **3.4a** and [ $^{18}\text{F}$ ]FENP **3.4b**. U/B = Uterus/Blood and U/M = Uterus/Muscle.<sup>120,121</sup>

The uptake of radioactivity in the uterus was rather constant in the investigated time span, but the ratios continued to increase, owing to a more rapid clearance of **3.4a,b** from the non-target tissues (Tables 3.5-6 and Figure 3.6). The uptake of radioactivity in bone was high, probably as a result of metabolic defluorination of **3.4a,b** and subsequent accumulation of [<sup>18</sup>F]fluoride in bone. The liver, which is known to be involved in steroid metabolism, also showed a high uptake. Furthermore, there is a considerable uptake of **3.4a,b** in fat, due to the lipophilic nature of the progestins.

From a qualitative analysis of the results, it can be concluded that little difference exists between **3.4a** and **3.4b**. The uptake of **3.4b** in the uterus is higher than **3.4a**, but, on the other hand, the accumulation of **3.4b** in fat is also somewhat higher than for **3.4a**. In order to select the most appropriate ligand, both **3.4a** and **3.4b** have been subjected to a distribution study in tumor-bearing rats and the metabolism of **3.4a,b** has been studied.<sup>52</sup> On the basis of these results, [<sup>18</sup>F]FENP **3.4b** has been selected for application in patients suffering from breast cancer or a meningioma.

### 3.5 Concluding remarks

The four 21-[<sup>18</sup>F]fluorine labelled progestins **3.4a,b,c,d** described in this Chapter have been prepared in satisfactory yields and with excellent specific activity. Two of these ligands, [<sup>18</sup>F]FMNP **3.4a** and [<sup>18</sup>F]FENP **3.4b** respectively, have been screened in an *in vivo* tissue distribution study, showing a highly selective and specific uptake in the target organ (i.e. the uterus). These results suggested that both compounds are potential tracers for the *in vivo* evaluation of PR. Indeed, the local Medical-Ethical Committee has approved the use of **3.4b** in the screening of patients suffering from breast cancer or a meningioma.

However, preliminary patient studies with **3.4b** failed to visualise a breast tumor that was known to contain both ER and PR.<sup>52</sup> [<sup>18</sup>F]FENP **3.4b** was also evaluated by Dehdashti *et al.*<sup>123</sup> in a group of patients with primary breast carcinomas. The authors concluded that **3.4b** was not suitable for the evaluation of PR-density in breast carcinomas, since the distribution of **3.4b** resulted in a low target/non-target ratio. It was suggested that the lipophilicity of **3.4b** and its metabolites was too high.

In a group patients with a meningioma uptake of **3.4b** was found in 2 out of 6 cases, but the uptake of **3.4b** seemed not to be correlated with the PR-density of

the tumor. The results of the patient studies with [ $^{18}\text{F}$ ]FENP **3.4b** have been discussed in detail in the thesis of Verhagen.<sup>52</sup>

### 3.6 Experimental part

*General remarks.*- Compounds **3.5b** and **3.4b** were gifts from Organon International, Oss, The Netherlands. Steroids **3.5a,c,d** were prepared by a method developed by van Leusen.<sup>118</sup> Tetrabutylammonium fluoride trihydrate (TBAF.3H<sub>2</sub>O) was dried by three successive evaporations with acetonitrile prior to use. Solvents were supplied by Janssen Chimica and kept on molecular sieves 4Å. The NMR-spectra were recorded in CDCl<sub>3</sub> unless otherwise stated. Chemical shifts are reported in  $\delta$  units (ppm) relative to TMS ( $^1\text{H}$  and  $^{13}\text{C}$ ) or CFC1<sub>3</sub> ( $^{19}\text{F}$ ). The splitting patterns are designed as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), d (double), br (broad). The [ $^{18}\text{F}$ ]fluoride was prepared and resolubilised with Kryptofix 222 (K<sub>222</sub>/ $^{18}\text{F}$ ) as described in Chapter 2. The pinch valves for the remote controlled system were supplied by Asco/Joucometic (Amersfoort, The Netherlands). The specific activity of the products was determined by measuring and comparing the UV-absorption of the labelled product and a known standard. Radioactive yields were determined in a dosis-calibrator.

*HPLC methods.*- The solvents used for HPLC were supplied by Merck (Darmstadt, FRG) and filtered and degassed before use. System A: a Waters radial-PAK silica Cartridge, 5 $\mu$ , 8x100 mm was eluted with hexane/dichloromethane/isopropanol 95/5/1 (v/v), flow 3 ml/min and used for routine purification. System B, a Waters radial-PAK C18 Cartridge eluted with acetonitrile/water 60/40 (v/v), flow 2 ml/min was used to check the identity and purity of the product. The eluent was monitored with a UV-detector set at 254 nm and a sodium iodide scintillation detector. Preparative HPLC was performed on a Chrompack silica column (15x250 mm) eluted with hexane/chloroform 70/30 (v/v), flow 5 ml/min.

*21-Tosylates 3.7a,b,c,d.*- To a solution of 300 mg (0.8 mmol) **3.5a,b,c,d** and 1.5 ml triethylamine in 20 ml CH<sub>2</sub>Cl<sub>2</sub> cooled in ice, was added 300 mg (1.5 mmol) *p*-toluenesulfonyl chloride. After 3 hours an additional amount of 150 mg *p*-toluenesulfonyl chloride was added. The solution was stirred for 3 h at 0 °C, then for another 3 h at room temperature. The reaction mixture was diluted with 30 ml CH<sub>2</sub>Cl<sub>2</sub> and washed twice with 20 ml 1 N HCl and twice with 20 ml of a saturated Na<sub>2</sub>CO<sub>3</sub>-solution. The organic layer was dried over MgSO<sub>4</sub> and the solvent was evaporated. The crude tosylates were purified on a silica column, eluted with CH<sub>2</sub>Cl<sub>2</sub>. Average yield was 300 mg (0.6 mmol, 75%) of **3.7a,b,c,d**.

**3.7a:**  $^1\text{H}$ -NMR (60 MHz)  $\delta$  0.7 (3H,s), 2.5 (3H,s), 0.6-2.9 (23H,m), 4.6 (2H,s), 5.9 (1H,s), 7.4 (2H,d), 7.9 (2H,d). Anal. (exact mass) C<sub>28</sub>H<sub>36</sub>O<sub>5</sub>S m/z 484.228, found 484.227.

**3.7b:**  $^1\text{H}$ -NMR  $\delta$  0.7 (3H,s), 2.4 (3H,s), 0.6-2.8 (25H,m), 4.5 (2H,s), 5.8 (1H,s), 7.3 (2H,d), 7.8 (2H,d). Anal. C<sub>29</sub>H<sub>38</sub>O<sub>5</sub>S m/z 498.244, found 498.243.

**3.7c:**  $^1\text{H-NMR}$   $\delta$  0.7 (3H,s), 1.2 (3H,s), 2.5 (3H,s), 0.6-2.6 (22H,m), 4.4 (2H,s), 5.8 (1H,s), 7.4 (2H,d), 7.9 (2H,d). Anal.  $\text{C}_{29}\text{H}_{38}\text{O}_5\text{S}$   $m/z$  498.244, found 498.244.

**3.7d:**  $^1\text{H-NMR}$   $\delta$  0.7 (3H,s), 1.2 (3H,s), 2.5 (3H,s), 0.6-2.6 (24H,m), 4.4 (2H,s), 5.8 (1H,s), 7.4 (2H,d), 7.9 (2H,d). Anal.  $\text{C}_{30}\text{H}_{40}\text{O}_5\text{S}$   $m/z$  512.260, found 512.259.

**21-Fluoro-progestins 3.4a,c,d.** - An amount of 100 mg (0.3 mmol) tetra-butylammonium fluoride trihydrate (TBAF.3H<sub>2</sub>O) was dried by three successive evaporations of 3 ml CH<sub>3</sub>CN. To the anhydrous TBAF was added 70 mg (0.15 mmol) of tosylate **3.7a,c,d**, dissolved in 5 ml CH<sub>3</sub>CN. The mixture was heated for 20 min on an oil-bath of 70 °C. A volume of 30 ml CH<sub>2</sub>Cl<sub>2</sub> was added and the organic layer was washed with 2 portions of 20 ml water. After drying over MgSO<sub>4</sub>, the solvent was evaporated and the crude product was purified on a silica column eluted with CH<sub>2</sub>Cl<sub>2</sub>. The average yield of **3.4a,c,d** was 30 mg (0.1 mmol, 30%). An analytically pure sample of **3.4a,c,d** was obtained after preparative HPLC.

**3.4a:**  $^1\text{H-NMR}$  (300 MHz)  $\delta$  0.73 (3H,s), 0.95 (3H,d), 0.8-2.5 (19H,m), 2.80 (1H,m), 4.73 (2H,d,  $^2J_{\text{HF}}=48\text{Hz}$ ), 5.82 (1H,s).  $^{13}\text{C-NMR}$  (75 MHz)  $\delta$  14.31 (q), 21.94 (q), 26.16 (t), 26.48 (t), 30.89 (t), 31.07 (d), 33.08 (t), 35.31 (t), 36.40 (t), 38.41 (t), 40.15 (d), 42.33 (d), 46.57 (s), 49.17 (d), 53.97 (d), 67.10 (d), 85.67 (dt,  $^1J_{\text{CF}}=186\text{Hz}$ ), 124.60 (d), 165.94 (s), 199.60 (s), 206.38 (s).  $^{19}\text{F-NMR}$  (200 MHz)  $\delta$  -224.86 (t,  $^2J_{\text{HF}}=48\text{Hz}$ ). Anal. (exact mass)  $\text{C}_{21}\text{H}_{29}\text{O}_2\text{F}$   $m/z$  332.215, found 332.215.

**3.4c:**  $^1\text{H-NMR}$   $\delta$  0.73 (3H,s), 0.95 (3H,d), 1.18 (3H,s), 1.0-2.5 (18H,m), 2.80 (1H,m), 4.74 (2H,d,  $^2J_{\text{HF}}=48\text{ Hz}$ ), 5.73 (1H,s).  $^{13}\text{C-NMR}$   $\delta$  14.25 (q), 17.28 (q), 20.77 (t), 21.94 (q), 31.10 (d), 31.71 (t), 32.63 (t), 33.25 (t), 33.83 (t), 35.37 (d), 35.59 (t), 38.44 (t), 38.4 (s), 46.39 (s), 53.47 (d), 54.68 (d), 66.99 (d), 85.64 (dt,  $^1J_{\text{CF}}=188\text{Hz}$ ), 123.85 (d), 170.51 (s), 199.19 (s), 206.36 (s).  $^{19}\text{F-NMR}$   $\delta$  -224.83 (t,  $^2J_{\text{HF}}=48\text{Hz}$ ). Anal.  $\text{C}_{22}\text{H}_{31}\text{O}_2\text{F}$   $m/z$  346.231, found 346.230.

**3.4d:**  $^1\text{H-NMR}$   $\delta$  0.70 (3H,s), 0.78 (3H,t), 1.15 (3H,s), 0.9-2.5 (20H,m), 2.6 (1H,m), 5.72 (1H,s), 4.73 (2H,d,  $^2J_{\text{HF}}=48\text{Hz}$ ), 5.72 (1H,s).  $^{13}\text{C-NMR}$   $\delta$  12.67 (q), 14.47 (q), 17.31 (q), 20.82 (t), 29.79 (t), 31.22 (t), 31.74 (t), 32.64 (t), 33.85 (t), 35.42 (d), 35.60 (t), 38.4 (s), 38.49 (t), 38.58 (d), 45.83 (s), 53.47 (d), 54.69 (d), 64.76 (d), 85.47 (dt,  $^1J_{\text{CF}}=188\text{Hz}$ ), 123.86 (d), 170.56 (s), 199.23 (s), 206.64 (s).  $^{19}\text{F-NMR}$   $\delta$  -224.67 (t,  $^2J_{\text{HF}}=48\text{Hz}$ ). Anal.  $\text{C}_{23}\text{H}_{33}\text{O}_2\text{F}$   $m/z$  360.246, found 360.245.

**21-[ $^{18}\text{F}$ ]Fluoroproggestins 3.4a,b,c,d.** - Using the remote controlled set-up as depicted in Figure 3.4, irradiated  $^{18}\text{O}$ -enriched water was pumped to vessel I and carefully distilled. The cap was removed by gently knocking the vessel, and an amount of 10 mg (26  $\mu\text{mol}$ ) Kryptofix 222 and 3 mg (9  $\mu\text{mol}$ )  $\text{K}_3\text{PO}_4 \cdot 7\text{H}_2\text{O}$ , dissolved in 1 ml acetonitrile/water 90/10 (v/v), was added through a tube to vessel I. The resulting  $\text{K}_{222}/^{18}\text{F}$  was dried by three successive evaporations of 0.3 ml CH<sub>3</sub>CN, resolubilised in 0.5 ml CH<sub>3</sub>CN, sonicated for 1 min and transported to vessel II by means of a peristaltic pump. Through a tube, 1 mg (2  $\mu\text{mol}$ ) tosylate **3.7a,b,c,d** dissolved in 0.1 ml CH<sub>3</sub>CN was added to the  $\text{K}_{222}/^{18}\text{F}$ . After 5 min at 70 °C, the mixture was transported into the reversed direction by the same pump via a silica Sep-Pak Cartridge to the rotary evaporator. The cartridge was eluted with 3 ml CH<sub>3</sub>CN. After evaporation of the



solvent, the crude product was dissolved in 0.5 ml eluent and injected on HPLC system A. The retention times of **3.4a,b,c,d** are listed in Table 3.3. Radiochemical yields were determined by HPLC and ranged from 7-14% (EOB). The total synthesis time was 100 min. The identity and specific activity of the product was determined by comparison with authentic material and ranged from 200-400 GBq/ $\mu$ mol (5,000-10,000 Ci/mmol, EOS).